Blood Levels of Organochlorines before and after Chemotherapy among Non-Hodgkin's Lymphoma Patients

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Abstract

Several small studies suggest a link between environmental exposure to organochlorine compounds and risk of non Hodgkin's lymphoma (NHL). Because NHL is uncommon, studies of the topic often use a population-based case-control design, in which cases generally are enrolled after treatment has begun. If chemotherapy affects blood levels of organochlorines, exposure will be misclassified and findings distorted. To determine whether chemotherapy alters serum levels of organochlorines in NHL cases, we compared serum samples before and after treatment in 22 cases diagnosed with NHL between March 1994 and August 1995 and enrolled in a clinical trial at the United States National Cancer Institute's Clinical Center. The time difference between pretreatment and posttreatment samples ranged from 15 to 27 months with an average of 20 months. Laboratory analyses were conducted in blinded pretreatment and posttreatment pairs of the subjects. Pretreatment and posttreatment organochlorine serum levels were compared using Pearson correlation coefficient (r) and paired t test. The pretreatment and posttreatment serum levels were highly correlated for 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE) and polychlorinated biphenyls (PCBs) PCB-138, PCB-153, PCB-156, and total PCBs (ranging from 0.78 to 0.93). Serum levels of all of these organochlorines significantly decreased between initiation and completion of chemotherapy, 25% for total PCB ($P = 0.0044$), 28% for DDE ($P = 0.0014$), 25% for PCB-138 ($P = 0.0053$), 27% for PCB-153 ($P = 0.0031$), and 29% for PCB-156 ($P = 0.045$). Neither weight change nor lipid change was correlated with changes in chemical levels. There was no association between the length of time between blood draws and changes in chemical levels. Our data raise the possibility that lymphoma treatment depresses serum organochlorine levels.

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Introduction

The incidence of NHL[2] has risen steadily in both developed and developing countries, long predating the epidemic of NHL related to AIDS (1). The causes of this increase are unknown (2). Some environmental and occupational chemical exposures have been investigated. For example, an association between agricultural exposure to DDT and risk of NHL has been observed in several questionnaire-based case-control studies (3–5). More recently, this hypothesis has been tested, using biological measures of DDE (the major metabolite of DDT) and other organochlorines. In a hospital-based case-control study of 28 NHL cases and 17 controls, Hardell *et al.*, (6) found that PCBs (but not DDE) measured in adipose tissue were associated with increased risk of NHL. In a nested case-control study of 74 NHL cases and 147 controls carried out in a general population-based cohort enrolled in 1974 and followed through 1994, Rothman *et al.*, (7) also found that PCBs, but not DDE, were associated with increased risk of NHL.

To assess the contribution of organochlorine exposure in the general population to risk of NHL, large studies are needed. Because DDT and PCB exposure in the general population cannot be assessed by questionnaire alone, biological measurement of these compounds is essential. Although case-control studies nested within prospective cohorts are ideal to assess this question, many cohort studies will not have a large enough number of NHL cases to be able to address this issue. Furthermore, if organochlorines contribute to the etiology of NHL, they may function as promoters via their suggested immune-modulating properties (8). If so, assessing exposure levels close to the time of NHL diagnosis becomes important, another limitation of cohort studies where most cases are diagnosed years after enrollment. Finally, disentangling the effects of PCBs, DDE, dioxins, and other organochlorines requires relatively large serum volumes that are generally unavailable in prospective cohort studies.

For all of these reasons, large population-based case-control studies of NHL will be a likely choice to study the organochlorine-NHL hypothesis. In many case-control studies, cases are enrolled after treatment has begun; therefore, it is critical to determine whether disease onset or treatment alters organochlorine levels. Disease effects can be assessed indirectly by studying organochlorine levels in a range of cases that vary by stage/grade and the presence or absence of clinical symptoms. Treatment effects can be assessed by studying patients before and after the initiation of chemotherapy.

We used a preexisting collection of paired serum samples from 22 cases to assess the effects of treatment on organochlorine levels.

[2] The abbreviations used are: NHL, non-Hodgkin's lymphoma; DDT, 2,2-bis-(p-chlorophenyl)-1,1,1-trichloroethane; DDE, 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene; PCB, polychlorinated biphenyl; NCI, National Cancer Institute.

Table 1 Descriptive statistics

	Mean	SD	Minimum	Maximum
Time difference between pretreatment and posttreatment samples (mo)	19.45	3.06	15	27
Age (pretreatment) (yr)	46.5	11.8	24	66

	Pretreatment		Posttreatment	
	Mean	SD	Mean	SD
Weight (kg)	80.18	13.53	84.14	15.85
Lipid (mg/ml)	5.83	1.38	6.20	1.77

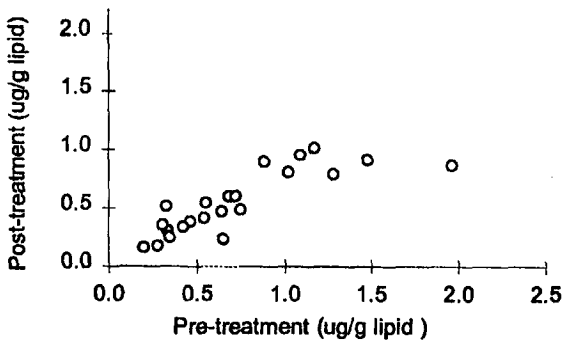

Fig. 1. Correlation between the lipid-adjusted serum levels of total PCBs among NHL patients (r = 0.84).

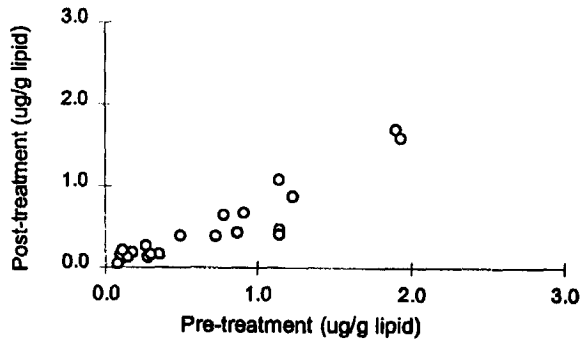

Fig. 2. Correlation between the lipid-adjusted serum levels of DDE among NHL patients (r = 0.93).

Materials and Methods

Patient Population. Eligible cases included 42 previously untreated men and women who were diagnosed with follicular, low-grade NHL between March 1994 and August 1995 and enrolled in a modified ProMACE chemotherapy (cyclophosphamide, doxorubicin, etoposide, and prednisone) clinical trial at the NCI Clinical Center. A total of 22 patients (6 women and 16 men) had available cryopreserved pretreatment and posttreatment serum samples of sufficient quantity for the analysis. All patients had signed the informed consent before entering the trial and Institutional Review Board approval was obtained from the NCI Clinical Center.

Assay Methods for DDE and PCBs. Randomly numbered serum samples were stored below −70°C. Laboratory analyses were conducted in blinded pretreatment and posttreatment pairs of the subjects assayed in the same batch. One ml of thawed serum was transferred by pipette to a centrifuge tube, and the volume of the serum was recorded to ±0.01 ml. Ten ng of a surrogate PCB (PCB-198) was added to the serum. One ml of methanol was added to denature albumin. The mixture was extracted three times with 5 ml of 50% ethyl ether:*n*-hexane, and the extracts were combined and concentrated to 10.0 ml. Two ml of this extract were removed for lipid determination. The remaining 8 ml were concentrated to 1 ml and transferred to 1 g of Florisil SPE column, and the pesticides and PCBs were eluted with 10 ml of *n*-hexane (fraction 1), followed by 10 ml of 1% ethyl ether in *n*-hexane (fraction 2). The fractions were concentrated to 1.0 ml, spiked with 10 ng of internal standard (PCB-119), and analyzed by gas chromatography with electron capture detection. Gas chromatography with electron capture detection was calibrated by repetitive analysis of standard mixtures of selected individual PCB congeners at six levels for the quantification of individual congener and total PCB levels. The analysis method for individual PCB congeners was a modified version of established methods (9–13). The total PCB level in each sample was also determined by summing the individual PCB congener levels. The procedure for determining the total PCB levels was validated by comparing to the Webb-McCall method (14).

We limited our analysis to PCB-138, PCB-153, and PCB-156 because these congeners were among those showing higher serum concentrations and a lower percentage of samples below the detection limit. The limit of detection was 0.2 ng/ml for DDE and 0.05 ng/ml for individual PCBs congeners and total PCBs. There were two subjects with DDE levels below the detection limit and six subjects with PCB-156 levels below the detection limit. For purposes of computation, we replaced the undetectable values by half of the values of the detection limits. The coefficients of variation for the DDE and PCB assays ranged from 35 to 49%.

Lipid Determination. One-g aluminum pans were embossed with labels and dried in an oven at 105°C for 1 h, allowed to cool in a desiccator, and then weighed to ±0.00001 g. Two ml of serum extract were transferred to each pan with *n*-hexane rinses. The pans were allowed to air dry, protected from dust, and then heated to 105°C and held at that temperature for 15 min. The pans were allowed to cool in a desiccator and then reweighed. Several empty (blank) pans were run simultaneously to check for method bias. The method for the total lipid analysis was adopted from the methods used by Sheldon (15, 16).

We calculated lipid-corrected values by dividing serum levels of chemicals by the total lipid value. To calculate the total lipid-corrected PCB levels, we divided each congener by the total lipid value and summed them.

Statistical Analysis. Pretreatment and posttreatment organochlorine serum levels were compared using Pearson correlation coefficient (r), paired t tests, and sign tests (17). Two sided Ps are reported for all comparisons.

Results

On an average, 20 months elapsed between the pretreatment and posttreatment blood draws (Table 1). There was a significant weight gain of ~5% (P = 0.012) and a nonsignificant increase in total lipid levels of ~6.5% (P = 0.17).

Table 2 Lipid-adjusted pretreatment *versus* posttreatment blood levels of DDE and PCBs among 22 NHL patients

Organochlorine	Mean ± SE		Difference[a]		Paired *t* test (P^b)	% change
	Pretreatment (μg/g lipid)	Posttreatment (μg/g lipid)	Mean ± SE	95% CI		
DDE	0.637 ± 0.125	0.458 ± 0.101	−0.18 ± 0.049	−0.28 to −0.078	−3.67 (0.0014)	−28.1
PCB-138	0.109 ± 0.082	0.082 ± 0.009	−0.027 ± 0.009	−0.045 to −0.009	−3.11 (0.0053)	−24.7
PCB-153	0.138 ± 0.019	0.100 ± 0.012	−0.038 ± 0.011	−0.061 to −0.027	−3.34 (0.0031)	−27.4
PCB-156	0.022 ± 0.005	0.016 ± 0.003	−0.006 ± 0.003	−0.013 to −0.0002	−2.14 (0.045)	−29.1
Total PCBs	0.731 ± 0.095	0.551 ± 0.058	−0.18 ± 0.056	−0.30 to −0.063	−3.19 (0.0044)	−24.6

[a] The difference between the posttreatment and pretreatment means. CI, confidence interval.
[b] Two-tailed.

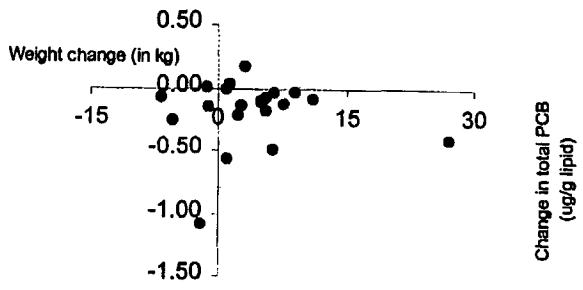

Fig. 3. Correlation between weight change and change in levels of lipid-adjusted total PCBs ($r = -0.01$).

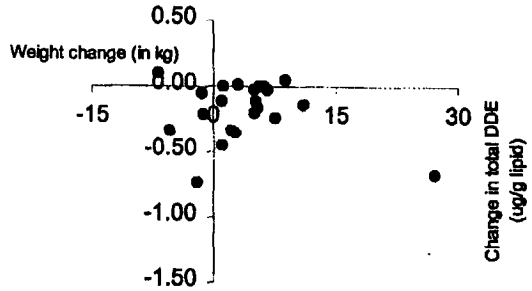

Fig. 4. Correlation between weight change and change in levels of lipid-adjusted DDE ($r = -0.21$).

The pretreatment and posttreatment levels were highly correlated for DDE, PCB-138, PCB-153, PCB-156, and total PCBs and correlation coefficients varied from 0.78 to 0.93. Figs. 1 and 2 show the correlation between the lipid-adjusted levels of pretreatment and posttreatment total PCBs and DDE, respectively. Lipid adjustments decreased the variability (*i.e.*, SD of PCB and DDE measurements were reduced) but did not appreciably alter the correlation coefficients. All subsequent analyses are shown with lipid adjustments.

As shown in Table 2, total PCBs fell from 0.73 μg/g lipid before treatment to 0.55 μg/g lipid after treatment overall. This change in total PCBs was significant ($P = 0.0044$ for paired *t* test and $P = 0.0009$ for sign test), with an average decrease (0.18 μg/g lipid) of 25%. Levels of significance were the same for paired *t* test with log transformation and sign test. The decline in DDE was also significant ($P = 0.0014$ by paired *t* test and $P = 0.0026$ by sign test), with an average decrease of 0.18 μg/g lipid (about a 28% decrease). The decline in PCB-138 was significant ($P = 0.0053$ by paired *t* test and $P = 0.0009$ by sign test), with an average decline of 0.027 μg/g lipid (about a 25% decrease). The decline in PCB-153 was significant ($P = 0.0031$ by paired *t* test and $P = 0.0043$ by sign test), with an average decline of 0.038 μg/g lipid (about a 27% decrease). The decline in PCB-156 was marginally significant ($P = 0.045$ by paired *t* test and $P = 0.021$ by sign test), with an average decrease of 0.006 μg/g lipid (about a 29% decrease).

We examined the effects of various possible cofactors on change in total PCB levels. The results were unaffected by adjustment for weight, age, sex, histological type, weight change, or change in lipid concentrations (Figs. 3 and 4). The decrease in total PCBs levels was largest for the stage 4 patients ($n = 16$; pretreatment, 0.82 μg/g lipid; posttreatment, 0.60 μg/g lipid; 26.8% decline) compared with the patients with stage 3 and below ($n = 6$; pretreatment, 0.48 μg/g lipid; posttreatment, 0.42 μg/g lipid; 12.5% decline); however, the difference was not significant ($P = 0.20$). The decrease in DDE levels was similar for the stage 4 patients (pretreatment, 0.76 μg/g lipid; posttreatment, 0.55 μg/g lipid; 27.6% decline) compared with the patients with stage 3 and below (pretreatment, 0.35 μg/g lipid; posttreatment, 0.25 μg/g lipid; 28.5% decline; $P = 0.23$).

Changes in levels of separate chemicals were highly correlated with each other (Table 3). The lower correlation coefficients for PCB-156 partly reflect the larger number of values below the level of detection (pre- and posttreatment values were both less than the detectable level for 6 of the 22 patients). Lipid change was uncorrelated with the changes in chemical levels. We found no evidence of an association between weight and the change in organochlorine levels ($P = 0.91$ for total PCBs and $P = 0.77$ for DDE). There was no association between the length of time between blood draws and the change in chemical levels.

Discussion

Pretreatment and posttreatment levels for DDE, PCB-138, PCB-153, PCB-156, and total PCBs were highly correlated among 22 patients. Posttreatment levels were significantly lower for each chemical, and the decline varied between 25 and 29%; the results were unaffected by adjustment for weight, age, sex, histological type, weight change, or change in lipid concentrations. Our observation of high correlation between pre- and posttreatment levels of PCBs and DDE agrees with the findings from a breast cancer study (18), but the 25% decrease we observed does not. In the breast cancer study, increases of serum levels of PCBs (29.4%) and DDE (15.8%) were observed among the chemotherapy group compared with the surgery group. The interval of observation was shorter (1–3 months) in the breast cancer study.

Table 3 Pearson correlation coefficients between changes in levels of DDE, PCBs, lipids, and weight

	DDE	PCB-138	PCB-153	PCB-156	Total PCBs	Lipid change	Weight change
DDE	1.00						
PCB-138	0.65	1.00					
PCB-153	0.72	0.91	1.00				
PCB-156	0.67	0.67	0.83	1.00			
Total PCBs	0.76	0.95	0.98	0.82	1.00		
Lipid change	−0.17	−0.04	−0.23	−0.20	−0.21	1.00	
Weight change	−0.21	0.00	0.00	0.17	−0.01	0.08	1.00

It is unlikely that secular trends could explain the decline observed during the interval between pretreatment and posttreatment blood sample collections, given that half-lives of these compounds are thought to be 5–30 years (19, 20). Moreover, we found no association between the length of time between blood draws and the change in chemical levels. Furthermore, a study of healthy women found little variation in serum samples collected over a 3-month interval (21), although this was shorter than the interval in our study.

In addition to the true biological elimination of the chemical, temporal changes in body weight may influence serum levels of PCBs and DDT because organochlorines are stored in adipose tissue. Our data show no association between weight change and decline in chemical levels.

The consistency of the decrease across all chemicals and the large magnitude of the decline suggest that chemotherapy may account for the change. With these data, it is not possible to determine whether values had already fallen, had risen, or stayed the same after diagnosis.

We were not able to look at intervening periods that precisely mimic the sample collection window of a population-based case-control study with rapid ascertainment (*i.e.*, <1 month after diagnosis). It would be instructive to follow cases over the entire treatment course with samples collected frequently to clarify when the decline occurs. We are currently following a series of cases from pretreatment over the course of chemotherapy with the second and third blood draws occurring within a few months, thereby determining whether we see a change in organochlorine levels during the time frame that is relevant for a population-based case-control study with rapid ascertainment. Although we did not have a control group in our study, we felt that it was important to publish this initial evaluation from a sample of convenience because of its unexpected results and to raise a cautionary note for people attempting to study the organochlorine-NHL relationship in a case-control study.

One could speculate that lymphoma itself increases serum organochlorine levels, which then revert to predisease levels among patients who respond to treatment. If the disease causes weight loss, this could release stored organochlorines into the blood stream and organochlorine levels would therefore be elevated. We cannot exclude this scenario because many of our cases had low-grade lymphomas and either partial or complete response to treatment. Our data did not allow us to examine directly a possible disease effect. To indirectly assess such an effect, one could evaluate levels in a range of untreated cases with different stages/grades.

Our study has several strengths. We took advantage of previously collected serum samples from a well-documented NCI clinical trial with a patient population that was fairly homogeneous in stage/grade level and treatment regimen. Furthermore we were able to examine the effect of weight change.

Overall, if there is no evidence of a disease effect and decline does not occur during the first few months of treatment (or is so highly consistent across cases that it can be adjusted for in the analysis), case-control studies may have a role in studying the organochlorine-NHL hypothesis. If these issues cannot be clearly resolved, then the resolution of this hypothesis will require large cohort studies.

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